Dose-dependent effects of cholecystokinin-8 on antpyloroduodenal motility, gastrointestinal hormones, appetite, and energy intake in healthy men

Ixchel M. Brennan,1,2 Tanya J. Little,1 Kate L. Feltrin,1 Andre J. P. M. Smout,3 Judith M. Wishart,1,2 Michael Horowitz,1,2 and Christine Feinle-Bisset1,2

1University of Adelaide Discipline of Medicine and 2National Health and Medical Research Council of Australia Centre of Clinical Research Excellence in Nutritional Physiology, Interventions, and Outcomes, Adelaide, South Australia, Australia; and 3Department of Gastroenterology and Hepatology, University Medical Centre, Utrecht, The Netherlands

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Horowitz M, Feinle-Bisset C. Dose-dependent effects of cholecystokinin-8 on antpyloroduodenal motility, gastrointestinal hormones, appetite, and energy intake in healthy men. Am J Physiol Endocrinol Metab 295: E1487–E1494, 2008. First published October 28, 2008; doi:10.1152/ajpendo.90791.2008.—CCK mediates the effects of nutrients on gastrointestinal motility and appetite. Intravenously administered CCK stimulates pyloric pressures, increases plasma PYY, and suppresses ghrelin, all of which may be important in the regulation of appetite and energy intake. The dose-related effects of exogenous CCK on gastrointestinal motility and gut hormone release, and the relationships between these effects and those on energy intake, are uncertain. We hypothesized that 1) intravenous CCK-8 would have dose-dependent effects on antpyloroduodenal (APD) pressures, plasma PYY and ghrelin concentrations, appetite, and energy intake and 2) the suppression of energy intake by CCK-8 would be related to the stimulation of pyloric motility. Ten healthy men (age 26 ± 2 yr) were studied on four separate occasions in double-blind, randomized fashion. APD pressures, plasma PYY and ghrelin, and appetite were measured during 120-min intravenous infusions of 1) saline (“control”) or 2) CCK-8 at 0.33 (“CCK0.33”), 3) 0.66 (“CCK0.66”), or 4) 2.0 (“CCK2.0”) ng kg⁻¹ min⁻¹. After 90 min, energy intake at a buffet meal was quantified. CCK-8 dose-dependently stimulated phasic and tonic pyloric pressures and plasma PYY concentrations (r > 0.70, P < 0.05) and reduced desire to eat and energy intake (r > −0.60, P < 0.05) without inducing nausea. There were relationships between basal pyloric pressure and isolated pyloric pressure waves (IPPW) with plasma CCK (r > 0.50, P < 0.01) and between energy intake with IPPW (r = −0.70, P < 0.05). Therefore, our study demonstrates that exogenous CCK-8 has dose-related effects on APD motility, plasma PYY, desire to eat, and energy intake and suggests that the suppression of energy intake is related to the stimulation of IPPW.

Brennan IM, Little TJ, Feltrin KL, Smout AJ, Wishart JM, Horowitz M, Feinle-Bisset C. Dose-dependent effects of cholecystokinin-8 on antpyloroduodenal motility, gastrointestinal hormones, appetite, and energy intake in healthy men. Am J Physiol Endocrinol Metab 295: E1487–E1494, 2008. First published October 28, 2008; doi:10.1152/ajpendo.90791.2008.—CCK mediates the effects of nutrients on gastrointestinal motility and appetite. Intravenously administered CCK stimulates pyloric pressures, increases plasma PYY, and suppresses ghrelin, all of which may be important in the regulation of appetite and energy intake. The dose-related effects of exogenous CCK on gastrointestinal motility and gut hormone release, and the relationships between these effects and those on energy intake, are uncertain. We hypothesized that 1) intravenous CCK-8 would have dose-dependent effects on antpyloroduodenal (APD) pressures, plasma PYY and ghrelin concentrations, appetite, and energy intake and 2) the suppression of energy intake by CCK-8 would be related to the stimulation of pyloric motility. Ten healthy men (age 26 ± 2 yr) were studied on four separate occasions in double-blind, randomized fashion. APD pressures, plasma PYY and ghrelin, and appetite were measured during 120-min intravenous infusions of 1) saline (“control”) or 2) CCK-8 at 0.33 (“CCK0.33”), 3) 0.66 (“CCK0.66”), or 4) 2.0 (“CCK2.0”) ng kg⁻¹ min⁻¹. After 90 min, energy intake at a buffet meal was quantified. CCK-8 dose-dependently stimulated phasic and tonic pyloric pressures and plasma PYY concentrations (r > 0.70, P < 0.05) and reduced desire to eat and energy intake (r > −0.60, P < 0.05) without inducing nausea. There were relationships between basal pyloric pressure and isolated pyloric pressure waves (IPPW) with plasma CCK (r > 0.50, P < 0.01) and between energy intake with IPPW (r = −0.70, P < 0.05). Therefore, our study demonstrates that exogenous CCK-8 has dose-related effects on APD motility, plasma PYY, desire to eat, and energy intake and suggests that the suppression of energy intake is related to the stimulation of IPPW.

The presence of nutrients in the small intestine is associated with the release of a number of gastrointestinal hormones, including cholecystokinin (CCK) and peptide YY (PYY) (12, 13, 30), and the suppression of ghrelin (34, 43), all of which modulate appetite and energy intake (4, 29, 44) and gastrointestinal motility (11, 29, 40). Of these hormones, CCK and its suppressing effects of CCK-8 on antpyloroduodenal (APD) motility and gut hormone release and the relationships between these effects with those on hunger and energy intake. The broad hypotheses were that 1) intravenous CCK-8 would have dose-dependent effects on these parameters and 2) the suppressive effects of CCK-8 on energy intake would be related to the stimulation of pyloric motility but independent of nausea.

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SUBJECTS AND METHODS

Subjects

Ten healthy males, aged 26 ± 2 yr (range 21–36 yr) and of normal body weight for their height (body mass index 23 ± 0.5 kg/m²; range 20–25 kg/m²), were recruited according to guidelines established by the Royal Adelaide Hospital Research Ethics Committee. All subjects were unrestricted eaters [score ≤12 on the eating restraint component of the Three-Factor Eating Questionnaire (42)]. The following exclusion criteria were also applied: 1) significant gastrointestinal disease, symptoms, or surgery; 2) current use of medication known to affect gastrointestinal function, appetite, or body weight; 3) significant cardiovascular or respiratory disease; 4) allergy to local anesthetic; 5) cigarette smoking or an alcohol intake in excess of 20 g/day; and 6) abnormal liver function and/or biochemistry tests. The protocol was approved by the Royal Adelaide Hospital Research Ethics Committee, and all experiments were carried out in accordance with the Declaration of Helsinki. All subjects provided informed, written consent prior to their enrolment.

Protocol

Each subject attended the laboratory on four occasions, each separated by 4–10 days, during which they received, in randomized, double-blind fashion, intravenous infusions of CCK-8 (Merck Biosciences, Läufelfingen, Switzerland) at either 1) 0.33 (“CCK0.33”), 2) 0.66 (“CCK0.66”), or 3) 2.0 (“CCK2.0”) ng·kg⁻¹·min⁻¹ or 4) isotonic saline (“control”). CCK-8 was dissolved in 0.9% sterile saline. In all studies, APD motility, plasma CCK, PYY and ghrelin concentrations, appetite, and energy intake were evaluated.

Subjects attended the laboratory at 0830 after fasting from solid and liquid food from 2200 the previous night. A 16-channel manometric catheter (Dentsleeve International), to measure pressures in the APD region, was inserted through an anesthetized nostril and allowed to pass through the pylorus into the duodenum by peristalsis (19). The catheter was positioned with six side holes in the antrum (channels 1–6), a 4.5-cm sleeve sensor (channel 7) with two side holes (channels 8 and 9) on the back of the sleeve, across the pylorus, and seven side holes in the duodenum (channels 10–16). The distance between side holes was 1.5 cm. The position of the catheter was maintained by measurement of the transmucosal potential difference between the most distal antral (channel 6, approximately –40 mV) and the most proximal duodenal (channel 10, ~0 mV) channel, using a cannula filled with sterile saline and placed subcutaneously in the left forearm as a reference electrode (19). All channels were perfused at 0.15 ml/min with degassed, distilled water, with the exception of the two transmucosal potential difference channels, which were perfused with degassed 0.9% saline (19). Intravenous cannulae were placed in each arm for the intravenous infusion and blood sampling, respectively.

Once the catheter was in place, fasting motility was monitored until the occurrence of a phase III of the interdigestive migrating motor complex, following which, and during a phase of motor quiescence, a baseline (t = −15 min) blood sample was taken and a visual analog scale (VAS) questionnaire, assessing perceptions of appetite (33), administered. At t = 0 min, infusion of either 1) control, 2) CCK0.33, 3) CCK0.66, or 4) CCK2.0 was commenced and continued for 120 min. During the infusion, blood samples were obtained and VAS completed at regular intervals, i.e., every 10 min between t = 0–30 min, every 15 min until t = 60 min, and every 30 min until t = 150 min. At t = 90 min, subjects were extubated and immediately offered a standardized, cold, buffet-style meal. The meal consisted of bread, cold meats, cheese, lettuce, tomato, cucumber, mayonnaise, butter, apple, banana, yogurt, chocolate custard, fruit salad, iced coffee, orange juice, and water, as described previously (14). The amount of food offered was in excess of what the subject was expected to consume. The subject was allowed ≤30 min to consume their meal and instructed to eat until comfortably full. At t = 120 min, the infusion was ceased. Subjects were then monitored for a further 30 min and, after removal of the intravenous cannulae, allowed to leave the laboratory.

Measurements

Antropyloroduodenal pressures. Manometric pressures were digitized and recorded on a computer-based system running commercially available software (HAD; Associate Prof. G. S. Hebbard, Royal Melbourne Hospital, Melbourne, Australia) and stored for subsequent analysis. APD pressures were analyzed for 1) the number and amplitude of pressure waves (PW) in the antrum and duodenum, 2) basal pyloric pressure (pyloric “tone”), and 3) the number and amplitude of IPPW. PW in the antrum, pylorus, and duodenum were defined by an amplitude ≥10 mmHg, with a minimum interval of 15 s between peaks for antral and pyloric waves and 3 s for duodenal waves, and analyzed using custom-written software (by A. Smout) (38). Basal pyloric pressures were calculated by subtracting the mean basal pressure (excluding phasic pressures) recorded at the most distal antral side hole from the mean basal pressure recorded at the mean (20), using custom-written software (by A. Smout).

Plasma hormone concentrations. Venous blood samples (10 ml) for evaluation of plasma CCK, PYY, and ghrelin were collected in ice-chilled EDTA-treated tubes containing 400 kIU aprotinin (Trasyol; Bayer Australia, Pymble, Australia) per liter blood. Plasma was separated by centrifugation (3,200 rpm, 15 min, 4°C) within 30 min of collection and stored at −70°C until assayed.

Plasma CCK concentrations (pmol/l) were determined by radioimmunoassay following ethanol extraction. A commercially available antibody (C258, lot 105H4852; Sigma-Aldrich, St. Louis, MO) raised in rabbits against synthetic sulfated CCK-8 was used. This antibody binds to all CCK peptides containing the sulfated tyrosine residue in position 7, shows a 26% cross-reactivity with unsulfated CCK-8 and <2% cross-reactivity with human gastrin, and does not bind to structurally unrelated peptides. Intra- and interassay coefficients of variation (CV) were 6.2 and 14.8%, respectively, with a detection limit of 2.5 pmol/l (39).

Plasma PYY concentrations (pmol/l) were determined by radioimmunoassay, using an antisem (kindly donated by Dr. B. Otto, Medizinische Klinik, Klinikum Innenstadt, University of Munich, Munich, Germany) raised in rabbits against human PYY1–36 (Sigma-Aldrich); i.e., the assay does not distinguish between PYY1–36 and PYY3–36. This antisem showed <0.001% cross-reactivity with human pancreatic polypeptide or sulfated CCK-8 and 0.0025% cross-reactivity with human neuropeptide Y. Tracer (NEX3410) was purchased from PerkinElmer (Boston, MA). Intra- and interassay CV were 12.3 and 16.6%, respectively, with a detection limit of 1.5 pmol/l (35).

Plasma ghrelin concentrations (pg/ml) were measured by an established radioimmunoassay, using a commercial antisem (RAST-4745; Bachem, Torrance, CA) that does not cross-react with human secretin, orexin, motilin, galanin, or vasoactive intestinal peptide. Intra- and interassay CV were 17 and 23%, respectively, with a detection limit of 40 ng/l (34).

Appetite and energy intake. Appetite ratings (desire to eat, fullness) were assessed using validated VAS (33). Nausea and bloating were also quantified. Each VAS consisted of a 100-mm horizontal line, where 0 mm represented “sensation not felt at all” and 100 mm represented “sensation felt the greatest.” The subject was asked to place a vertical mark along the line to indicate the strength of each sensation.

The amount (g) of food consumed from the buffet meal was determined by weighing the meal before and after consumption. Energy intake (kJ) and macronutrient composition (%energy from fat, carbohydrate, and protein) were analyzed using commercially available software (Foodworks 3.01; Xyris Software, Highgate Hill, QLD, Australia) (7).
Statistical Analysis

Baseline values (‘0’) were calculated as the mean of values obtained at \( t = -15 \) and 0 min for VAS and plasma hormone concentrations and between \( t = -15 \) and 0 min for the total number and mean amplitude of antral and duodenal PW, IPPW, and basal pyloric pressures. The number and amplitude of antral and duodenal PW were expressed as total and mean values, respectively, during the first 90 min of the infusion period. IPPW and basal pyloric pressure were expressed as mean values of 15-min intervals between 0 and 90 min (i.e., 0–15, 15–30, 30–45, 45–60, 60–75, and 75–90 min). All data, with the exception of plasma hormone concentrations, were expressed as changes from baseline. VAS, plasma hormone concentrations, IPPW, and basal pyloric pressures were analyzed by repeated-measures ANOVA, with time and treatment as factors. Areas under the curves (AUCs) for basal pyloric pressure, number, and amplitude of IPPW and plasma hormone concentrations were determined using the trapezoidal rule. The number and amplitude of antral and duodenal PW and energy intake were analyzed by one-way ANOVA. Post hoc paired comparisons, adjusted for multiple comparisons by Bonferroni’s correction, were performed when ANOVAs revealed significant effects. Plasma hormone concentrations at \( t = 90, 120, \) and 150 min were compared using Student’s \( t \)-test. Correlations, corrected for repeated measures, were determined for 1) the total number and mean amplitude of antral and duodenal PW, AUCs (between \( t = 0 \) and 90 min) for basal pyloric pressures, number and amplitude of IPPW and plasma hormone concentrations, and energy intake with the natural logarithm-transformed CCK-8 doses; and 2) energy intake with AUCs for APD pressures and plasma hormone concentrations using the method described by Bland and Altman (6). Only \( r \) values > 0.5 were considered physiologically relevant. Statistical significance was accepted at \( P < 0.05 \), and data are presented as means ± SE.

RESULTS

All subjects completed the four randomized study days and tolerated the experimental conditions well.

Antropyloroduodenal Pressures

**Antral pressures.** There was a significant effect of treatment on both the number and amplitude of antral PW (\( P < 0.01 \) for both; Table 1). The number of antral PW was lower during both CCK0.66 and CCK2.0 when compared with control (\( P < 0.01 \) for both), with no difference between CCK0.33 and control, and lower during CCK2.0 when compared with CCK0.33 (\( P < 0.05 \)), with no difference between CCK2.0 and CCK0.66. The amplitude of antral PW was lower during all three treatments when compared with control (\( P < 0.001 \) for all), with no differences between treatments (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Number and amplitude of antral, pyloric, and duodenal pressure waves during 90-min intravenous infusion of saline (control) or CCK-8 at CCK0.33, CCK0.66, and CCK2.0</th>
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<tr>
<td>CCK-8, ng·kg(^{-1})·min(^{-1})</td>
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<tr>
<td>-----------------------------------------------</td>
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<tr>
<td><strong>Antral pressure waves</strong></td>
</tr>
<tr>
<td>No.</td>
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<tr>
<td>Amplitude, mmHg</td>
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<td><strong>Duodenal pressure waves</strong></td>
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<td>No.</td>
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<td>Amplitude, mmHg</td>
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Data are means ± SE (\( n = 10 \)). CCK, cholecystokinin. CCK0.33, CCK0.66, and CCK2.0, 0.33, 0.66, and 2.0 ng·kg\(^{-1}\)·min\(^{-1}\), respectively. *Vs. control, \( P < 0.05 \); #vs. CCK0.33, \( P < 0.05 \).

**Pyloric pressures.** Basal pressure (tone). There was a treatment-by-time interaction for basal pyloric pressure (\( P < 0.01 \); Fig. 1A). Basal pyloric pressure rose within 15 min of the start of the CCK infusions (\( P < 0.05 \)) and was higher during CCK0.33 between \( t = 15 \) and 90 min (\( P < 0.05 \)), during CCK0.66 between \( t = 30 \) and 90 min (\( P < 0.05 \)), and during CCK2.0 between \( t = 0 \) and 90 min (\( P < 0.01 \)) when compared with control. Basal pyloric pressure was also higher during CCK2.0 between \( t = 60 \) and 90 min when compared with CCK0.33 (\( P < 0.05 \)) and between \( t = 45 \) and 90 min when compared with CCK0.66 (\( P < 0.05 \)).

**PHASIC PRESSURES.** There was a treatment-by-time interaction for the number of IPPW (\( P < 0.001 \); Fig. 1B). The number of IPPW reached a peak at 15–30 min during all CCK infusions, after which time the response declined (\( P < 0.01 \)). The number was lower during all three treatments when compared with control (\( P < 0.001 \) for all), with no differences between treatments (Table 1).
of IPPW was higher during CCK0.66 and CCK2.0 between \( t = 0 \) and 90 min \( (P < 0.01 \) for both), and during CCK0.33 between \( t = 0 \) and 45 min and \( t = 60 \) and 75 min \( (P < 0.05) \), when compared with control. The number of IPPW was higher during CCK0.66 between \( t = 0 \) and 30 min and \( t = 60 \) and 75 min \( (P < 0.05) \), when compared with CCK0.33, and during CCK2.0 between \( t = 0 \) and 30 min and \( t = 45 \) and 90 min \( (P < 0.05) \), when compared with CCK0.33, and between \( t = 30 \) and 45 min \( (P < 0.001) \) when compared with CCK0.66.

There was an effect of treatment on the amplitude of IPPW \( (P < 0.001) \), which was higher during all three treatments when compared with control \( (P < 0.001 \) for all), with no differences between treatments (mean values between \( t = 0 \) and 90 min: control, \( 11 \pm 3 \) mmHg; CCK0.33, \( 31 \pm 6 \) mmHg; CCK0.66, \( 36 \pm 5 \) mmHg; CCK2.0, \( 38 \pm 7 \) mmHg).

Duodenal pressures. There was a significant effect of treatment on both the number and amplitude of duodenal PW \( (P < 0.05 \) for both; Table 1). The number of duodenal PW was lower during both CCK0.66 and CCK2.0 when compared with control \( (P < 0.01 \) for both), with no difference between CCK0.33 and control or between CCK0.33, CCK0.66, and CCK2. The amplitude of duodenal PW was lower during CCK2.0 when compared with control \( (P < 0.01) \), with no difference between CCK0.33 or CCK0.66 and control or between CCK0.33, CCK0.66, and CCK2 (Table 1).

Gastrointestinal Hormone Concentrations

**Plasma CCK.** There was no difference in baseline CCK-8 concentrations between study days.

**Effect of Intravenous Infusion.** There was a treatment-by-time interaction for plasma CCK concentrations \( (P < 0.001; \) Fig. 2A). Plasma CCK remained at baseline concentrations during the control infusion and was higher during all three treatments when compared with baseline \( (P < 0.001) \). Plasma CCK concentrations were higher during all three treatments between \( t = 10 \) and 90 min when compared with control \( (P < 0.001) \), during CCK0.66 at \( t = 90 \) min when compared with CCK0.33 \( (P < 0.05) \), and during CCK2.0 between \( t = 10 \) and 90 min when compared with both CCK0.33 and CCK0.66 \( (P < 0.001 \) for both).

**Effect of Meal.** Immediately after the buffet meal (i.e., \( t = 120 \) min), plasma CCK concentrations were higher following control \( (P < 0.001) \), and lower following CCK0.66 and CCK2.0 \( (P < 0.05 \) for both), when compared with premeal concentrations (i.e., \( t = 90 \) min). At \( t = 150 \) min, plasma CCK concentrations were higher following control and lower following CCK0.66 and CCK2.0 \( (P < 0.05 \) for all) when compared with concentrations at \( t = 90 \) min, whereas there was no difference between treatments.

**Plasma PYY.** There was no difference in baseline PYY concentrations between study days.

**Effect of Intravenous Infusion.** There was a treatment-by-time interaction for plasma PYY concentrations \( (P < 0.01; \) Fig. 2B). Plasma PYY remained at baseline concentrations during control and CCK0.33 and was higher during CCK0.66 and CCK2.0 when compared with baseline values \( (P < 0.01) \). Plasma PYY concentrations were higher during both CCK0.66 and CCK2.0, reaching significance during CCK0.66 at \( t = 90 \) min when compared with control \( (P < 0.001) \) and during CCK2.0 between \( t = 30 \) and 90 min when compared with control and CCK0.33 \( (P < 0.01 \) for both) and between \( t = 10 \) and 30 min when compared with CCK0.66 \( (P < 0.05) \).

**Effect of Meal.** Immediately after the buffet meal (i.e., \( t = 120 \) min), and at \( t = 150 \) min, plasma PYY concentrations were higher following all infusions \( (P < 0.01) \) when compared with premeal concentrations (i.e., \( t = 90 \) min), whereas there was no difference between treatments.

**Plasma ghrelin.** Plasma ghrelin was analyzed in only six subjects. There was no difference in baseline ghrelin concentrations between study days.

**Effect of Intravenous Infusion.** There was a treatment-by-time interaction for plasma ghrelin concentrations \( (P < 0.01; \) Fig. 2C). Plasma ghrelin concentrations were higher following all infusions \( (P < 0.01 \) for both) when compared with baseline concentrations \( (P < 0.05 \) for both; Table 1). Plasma ghrelin remained at baseline concentrations during the control infusion (i.e., \( t = 120 \) min), and at \( t = 150 \) min, plasma ghrelin concentrations were higher following all infusions \( (P < 0.01) \) when compared with premeal concentrations (i.e., \( t = 90 \) min), whereas there was no difference between treatments.

**Effect of Meal.** Immediately after the buffet meal (i.e., \( t = 120 \) min), and at \( t = 150 \) min, plasma ghrelin concentrations were higher following all infusions \( (P < 0.01) \) when compared with premeal concentrations (i.e., \( t = 90 \) min), whereas there was no difference between treatments.
Fig. 2C). Plasma ghrelin concentrations increased slightly during the control infusion and were lower during all three treatments when compared with baseline values ($P < 0.01$). Plasma ghrelin concentrations were lower during CCK0.33 between $t = 10$ and $90$ min ($P < 0.05$), during CCK0.66 and CCK2.0 between $t = 30$ and $90$ min ($P < 0.01$) when compared with control, and during CCK2.0 between $t = 30$ and $90$ min ($P < 0.05$) when compared with CCK0.66.

**EFFECT OF MEAL.** Immediately after the buffet meal (i.e., $t = 120$ min), there was no difference between treatments in plasma ghrelin concentrations when compared with premeal concentrations (i.e., $t = 90$ min). At $t = 150$ min, plasma ghrelin was lower following control, CCK0.66, and CCK2.0 ($P < 0.05$ for all), but not CCK0.33, when compared with concentrations at $t = 90$ min, whereas there was no difference between treatments.

**Appetite**

There was a treatment-by-time interaction for desire to eat ($P < 0.001$; Fig. 3). Desire to eat continued to increase during control and decreased during CCK2.0 over the entire infusion period when compared with baseline values ($P < 0.01$ for both), with no changes occurring during CCK0.33 and CCK0.66. Desire to eat was less during CCK0.33 between $t = 20$ and $30$ min and $t = 60$ and $90$ min ($P < 0.01$), during CCK0.66 between $t = 20$ and $90$ min ($P < 0.05$), and during CCK2.0 between $t = 10$ and $90$ min ($P < 0.05$) when compared with control. Desire to eat was less during CCK2.0 at $t = 30$ min and between $t = 60$ and $90$ min when compared with both CCK0.33 and CCK0.66 ($P < 0.05$ for both).

There was a significant effect of treatment on fullness ($P < 0.05$; data not shown). Fullness was higher during CCK0.33, CCK0.66, and CCK2.0 when compared with control ($P < 0.05$ for all), with no difference between treatments.

There was no effect of treatment on nausea, which increased <8% from baseline. There was a significant effect of time, but not treatment, on bloating (time effect: $P < 0.05$) (data not shown). Bloating was ~10% higher than baseline scores during CCK2.0 between $t = 0$ and $20$ min and $t = 45$ and $90$ min when compared with baseline ($P < 0.05$).

**Energy Intake**

There was a significant effect of treatment on both the amount eaten (g; $P < 0.05$) and energy intake (kJ; $P < 0.01$) at the buffet meal (Table 2). The amount eaten was less during CCK0.66 and CCK2.0 when compared with control and CCK0.33 ($P < 0.05$ for both), with no differences between CCK0.33 and control or between CCK0.66 and CCK2.0. Energy intake was less during CCK2.0 when compared with control ($P < 0.01$), CCK0.33 ($P < 0.01$), and CCK0.66 ($P < 0.05$), with no significant differences between CCK0.33, CCK0.66, and control or between CCK0.33 and CCK0.66.

**Relationships Between Antropyloroduodenal Motility, Plasma Hormones, Appetite, and Energy Intake With the Dose of CCK-8 Administered**

There were direct relationships between basal pyloric pressure ($r = 0.80, P < 0.01$), the number of IPPW ($r = 0.70, P < 0.01$), plasma CCK ($r = 0.80, P < 0.01$), and plasma PYY ($r = 0.70, P < 0.05$) and inverse relationships between desire to eat ($r = -0.60, P < 0.05$) and the amount of food ($r = -0.70, P < 0.05$) and energy ($r = -0.70, P < 0.01$) consumed at the buffet meal, with the dose of CCK-8 administered.

**Relationships Between Antropyloroduodenal Motility With Plasma Hormones**

There were direct relationships between both basal pyloric pressure ($r = 0.50, P < 0.01$) and the number of IPPW ($r = 0.60, P < 0.01$) with plasma CCK and between the number of IPPW with plasma PYY ($r = 0.60, P < 0.05$).

**Relationships Between Energy Intake With Antropyloroduodenal Motility and Plasma Hormones**

There was an inverse relationship between energy intake with the number of IPPW ($r = -0.70, P < 0.05$) but no other motility parameter or gastrointestinal hormones.

**DISCUSSION**

Our study is the first to comprehensively evaluate the dose-related effects of CCK-8 on APD motility, gastrointestinal hormone release, appetite, and energy intake. The observations establish that exogenous administration of CCK-8, at the doses evaluated, has discrepant effects on APD motility, PYY and ghrelin, appetite, and energy release.
intake in healthy male subjects and that energy intake is inversely related to the stimulation of IPPW.

Clarification of the dose-related effects of CCK-8 is relevant for a greater understanding of the mechanisms relating to the regulation of APD motility, PYY and ghrelin, and appetite and energy intake. Healthy young males were studied since they have been reported to have a greater capacity to adjust energy intake in response to dietary manipulation when compared with elderly men, healthy females, or obese individuals (37, 41), and the number of subjects included was based on power calculations derived from our previous studies (7, 13, 29). The doses of CCK-8 were selected on the basis of previous studies, in which CCK-8 at a dose of 2 ng·kg\(^{-1}\)·min\(^{-1}\) resulted in moderately supraphysiological plasma concentrations and was shown to have submaximal effects on APD motility and energy intake (7, 29). That the doses of CCK-8 used in this study were physiological is also supported by the postprandial concentrations observed.

Intravenous CCK-8 modulates gastroduodenal motility and slows gastric emptying (15, 31). This study has demonstrated for the first time that CCK-8 at a dose as low as 0.33 ng·kg\(^{-1}\)·min\(^{-1}\) stimulates pyloric PW, whereas higher doses of CCK-8, i.e., 0.66 and 2.0 ng·kg\(^{-1}\)·min\(^{-1}\), were required for suppression of antral and duodenal PW. These observations indicate that there are differences in the sensitivity of the APD region to CCK. Some studies conducted in animals support these findings (18, 22). For example, regional heterogeneity of CCK receptors on smooth muscle and neurons in the gut has been observed in the guinea pig (18). In humans, CCK released from the small intestine acts directly at CCK-1 receptors, which are expressed by vagal afferent neurons of the stomach and small intestine. Studies employing the specific CCK-1 receptor antagonist loxiglumide have established that endogenous CCK has a physiological role in the regulation of gastrointestinal motility (16, 17).

Following the ingestion of a meal, a number of peptides other than CCK are released, including PYY (1) and GLP-1 (21), and ghrelin is suppressed (43). There is evidence of interactions between these hormones that may enhance their effects on gastrointestinal motor function and energy intake. For example, intravenous PYY suppresses ghrelin in humans (3), and in vitro studies in rodents suggest that GLP-1 also has this effect (28). In dogs, the stimulation of PYY by the presence of fat in the proximal small intestine is mediated, at least in part, by CCK (27). Conversely, there is evidence in humans that GLP-1 may inhibit the release of PYY (32). Our recent study in healthy men demonstrated that exogenous CCK-8, but not GLP-1, markedly stimulated the release of PYY and suppression of ghrelin (8), supporting work of others (9, 27). The current study confirms and extends these findings by demonstrating that the effect of exogenous CCK-8 on plasma PYY concentrations is dose dependent. A previous study demonstrated that the fat-induced suppression of ghrelin release was reversed, and the fat-induced stimulation of PYY abolished, by the CCK-1 receptor antagonist deloxiglumide, suggesting that both effects are mediated via the CCK-1 receptor (9). As expected, the buffet meal stimulated an increase in plasma PYY concentrations, and although mean plasma ghrelin concentrations were less following the meal, this reduction was not statistically significant, which may well reflect the smaller sample size (i.e., \(n = 6\)). The observation that CCK affects other gastrointestinal hormones provides additional insight into potential mechanisms through which CCK modulates gut motility, appetite, and energy intake.

It appears that the acute effects of endogenous CCK on energy intake are more modest and require significantly more subjects, compared with exogenous CCK, that more frequently result in “supraphysiological” plasma concentrations. For example, 40 healthy subjects were included in a study that intravenously infused loxiglumide for 1 h prior to, and during, ingestion of a mixed nutrient meal and demonstrated an \(-10\%\) increase in energy intake when compared with the control infusion (5). In contrast, exogenous administration of CCK-8 potently increases fullness and suppresses hunger and energy intake, even in studies with small subject numbers (\(n = 8-12\)) (7, 23, 29). However, only few studies have demonstrated that CCK-8, when infused intravenously to produce “physiological” plasma concentrations, i.e., concentrations comparable with those observed after a mixed-nutrient meal in humans, suppresses energy intake (2, 25). In the current study, involving 10 healthy subjects, CCK2.0 markedly reduced energy intake by 20% when compared with control, comparable in magnitude with that reported by Ballinger et al. (2). Since exogenous administration of CCK can induce nausea, possibly as a result of elevated plasma CCK concentrations, the potent suppression of appetite and energy intake has been attributed to nausea. In our study, intravenous CCK reduced appetite and energy intake in the absence of nausea. It should be recognized that the absence of a significant effect of the lower doses of CCK-8 on energy intake may reflect the timing of the meal. Since CCK is known to be released during meal ingestion, and changes in basal pyloric pressure, IPPW, and plasma hormone concentrations were all observed to peak within \(\sim 30\) min after commencement of the CCK-8 infusions, individuals may have consumed less energy if the buffet meal had been consumed earlier, e.g., at \(t = 30\) min rather that \(t = 90\) min.

In dogs, electrical stimulation of the pylorus, increasing both tonic and phasic pressures, has been reported to be associated with suppression of energy intake (45). Given the association between energy intake and pyloric motility demonstrated in our previous work (7), in the current study we anticipated that changes in energy intake would be related to a greater modulation of antropyloroduodenal motility, particularly pyloric pressures. That there was an inverse relationship between energy intake and the number of isolated pyloric PW, i.e., the reduction in energy intake was associated with the stimulation of pyloric motility, is consistent with this concept. This suggests that an individual in whom there is greater stimulation of IPPW from a given stimulus should eat less, potentially be associated with small intestinal feedback is greater. Certainly, any effects of CCK on gastrointestinal motility that are relevant to its appetite-suppressant properties warrant further investigation.

In conclusion, this study has demonstrated that, in healthy males, CCK-8 stimulates pressures in the pylorus, increases plasma PYY concentrations, and suppresses desire to eat and energy intake in a dose-dependent manner, whereas all CCK-8 doses equally suppressed ghrelin. There were relationships between plasma CCK with basal pyloric pressure and IPPW and energy intake with IPPW. Hence, these data provide evidence that enhanced stimulation of IPPW is associated with suppression of energy intake. In view of this, the relationship between energy intake and pyloric motility, as well as the combination of CCK
with other peptides to suppress appetite and potentially induce weight loss, warrants further investigation.

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